PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6: A01N 63/04, 63/00

A1

(11) International Publication Number: WO 96/39844

(43) International Publication Date: 19 December 1996 (19.12.96)

(21) International Application Number: PCT/US96/09950

(22) International Filing Date: 7 June 1996 (07.06.96)

(30) Priority Data:
08/475,922 7 June 1995 (07.06.95) US

(71) Applicant: MYCOGEN CORPORATION [US/US]; 5501 Oberlin Drive, San Diego, CA 92121 (US).

(72) Inventors: JOHNSON, David; 1399 Woodlynn Avenue, Roseville, MN 55113 (US). JONES, Keith; 15662 Bernardo Center Drive #1210, San Diego, CA 92127 (US). WYSE, Donald, L.; 26370 Golden Court, Wyoming, MN 55092 (US).

(74) Agents: SANDERS, Jay, M. et al.; Saliwanchik & Saliwanchik, Suite A-1, 2421 N.W. 41st Street, Gainesville, FL 32606-6669 (US).

(81) Designated States: AU, BR, CA, JP, KR, MX, NZ, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: MATERIALS AND METHODS FOR INOCULATING PLANTS WITH MICROBES

(57) Abstract

The subject invention pertains to materials and compositions for applying live microbes to aerial plant portions such that the microbes will colonize and occupy the plant surface. The microbes are applied as part of an oil-in-water emulsion. The microbes may be, for example, a bacteria, fungi, or yeast. The microbes used according to the subject invention may be herbicidal, pesticidal, or may modulate plant growth or development.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	**GB**	United Kingdom	**MW**	Malawi
AT	Austria	**GE**	Georgia	**MX**	Mexico
AU	Australia	**GN**	Guinea	**NE**	Niger
BB	Barbados	**GR**	Greece	**NL**	Netherlands
BE	Belgium	**HU**	Hungary	**NO**	Norway
BF	Burkina Faso	**IE**	Ireland	**NZ**	New Zealand
BG	Bulgaria	**IT**	Italy	**PL**	Poland
BJ	Benin	**JP**	Japan	**PT**	Portugal
BR	Brazil	**KE**	Kenya	**RO**	Romania
BY	Belarus	**KG**	Kyrgystan	**RU**	Russian Federation
CA	Canada	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CF	Central African Republic			**SE**	Sweden
CG	Congo	**KR**	Republic of Korea	**SG**	Singapore
CH	Switzerland	**KZ**	Kazakhstan	**SI**	Slovenia
CI	Côte d'Ivoire	**LI**	Liechtenstein	**SK**	Slovakia
CM	Cameroon	**LK**	Sri Lanka	**SN**	Senegal
CN	China	**LR**	Liberia	**SZ**	Swaziland
CS	Czechoslovakia	**LT**	Lithuania	**TD**	Chad
CZ	Czech Republic	**LU**	Luxembourg	**TG**	Togo
DE	Germany	**LV**	Latvia	**TJ**	Tajikistan
DK	Denmark	**MC**	Monaco	**TT**	Trinidad and Tobago
EE	Estonia	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**UG**	Uganda
FI	Finland	**ML**	Mali	**US**	United States of America
FR	France	**MN**	Mongolia	**UZ**	Uzbekistan

DESCRIPTION

MATERIALS AND METHODS FOR INOCULATING PLANTS WITH MICROBES

Field of the Invention

The present invention relates to the use of oil-in-water emulsions to deliver live microbes to plants. The use of oil-in-water emulsions enhances the ability of the microbes to colonize and occupy the aerial plant portions.

Background of the Invention

For decades, researchers and commercial entities have been attempting to use live microbial agents as horticultural tools to kill unwanted vegetation, control pests, or enhance plant growth. There have been technical and commercial successes in selected applications. These successes include inoculants of root-associated nitrogen fixing bacteria; some plant growth promoting or protective treatments with seed-, root-, and soil-inhabiting bacteria and fungi; and foliar treatments with bacteria such as *Bacillus thuringiensis* which contain insecticidal proteins. In the case of *Bacillus thuringiensis*, the viability of the microbe is not required as evidenced by the success of applying killed bacteria expressing the insecticidal toxin.

Live biocontrol and plant growth-enhancing organisms have achieved some success in soil applications, but success in foliar applications has been very limited. Some progress has been made in the special case of post-harvest treatment of produce because, in this specialized setting, the environment in which the applied microbe must function can be controlled.

Starting in the early 1980s a significant research effort was directed at the development f inundative mycoherbicides—plant pathogenic fungi which could be used to control weeds. Naturally-occurring fungi with desirable selectivity for weeds among desirable crop species have been identified. See, for example, U.S. Patent Nos. 4,776,873; 4,755,208; 4,755,207; and 5,256,627. However, after many years and large expenditures, the success of mycoherbicides has been limited to a few special situations in which the environment consistently provides the moisture necessary for natural fungus infection, most notably in rice paddies. In general, the aerial surfaces of plants have proven to be an environment which is too harsh and variable for predictable inoculation with microbes. Researchers have attempted to address this limitation through formulations containing humectant materials or which "encapsulate" water, as in the case of invert emulsions. Also, muciloid compositions have been used to facilitate fungal bioherbicide activity. See U.S. Patent No.

5,393,728. Though some technical success has been reported, none of these technologies has translated into practical agricultural or horticultural use.

Bacterial agents have also been studied for control of insects and plant disease. Bacteria had initially been ruled out as bioherbicide candidates because bacterial pathogens are generally not capable of infecting plants directly—they typically require a wound or some other means of entry into the plant. This limitation has been overcome to some extent by the development of a biocontrol agent for annual bluegrass control originally discovered by Roberts *et al.*, U.S. Patent No. 5,077,045. In this case, isolates of the bacterium *Xanthomonas campestris* are introduced into the weed during the mowing process in managed turf where annual blue grass is a problem. The bacteria, once introduced into the xylem through the wound, causes a highly selective wilt disease of the annual bluegrass. This means of circumventing the variability of the external, aerial plant environment is very attractive; however, in most settings where living microbes could be of benefit, wounding-inoculation is not a practical alternative. Subsequently, further examples of the use of *Xanthomonas* as bioherbicides have been reported. See, for example, U.S. Patent No. 5,192,541.

Zidak *et al.* showed that by using certain silicone surfactants it is possible to introduce bacteria directly into a plant through stomatal openings. The silicone surfactants produce solutions with extremely low surface tensions, far lower than those produced by most other classes of surfactants. This low surface tension allows the liquid applied to a plant to flood stomata—something that water will not normally do on a plant surface. This approach has been used to apply the toxigenic bacterium *Pseudomonas syringae* pv. *tagetis* to Canada thistle in the absence of dew or mechanical wounding.

As promising as the silicon surfactant-based technology has become, it has certain limitations. The surfactants themselves are very expensive, have a narrow safety margin in terms of burning damage on desirable crops, and are subject to hydrolysis in the spray tank. It has not been possible to circumvent these limitation using alternate surface-active materials.

Brief Summary of the Invention

The subject invention pertains to the use of hydrophobic liquid oils emulsified in water as carriers for microbes to be applied to plants. The hydrophobic oils which can be used in the compositions and methods of the subject invention include, but are not limited to, paraffinic oils, vegetable- and animal-based oils (triglycerides), and derivatives thereof such as methyl esters of fatty acids. In a preferred embodiment, these hydrophobic compounds are combined with sufficient levels of a suitable emulsifier(s) to allow emulsification in water. The preparation of these solutions is easily accomplished by a person skilled in the art using the teachings provided herein. The desired

microbial agent is suspended in this emulsion at the time of application and sprayed or drenched onto plant surfaces.

Microbial agents that can be used according to the subject invention include, but are not limited to, *Bacillus* sp., *Pseudomonas* sp., *Xanthomonas, Trichoderma* sp., *Erwinia* sp., *Pichia* sp., *Candida* sp., *Cryptococcus* sp., *Talaromyces* sp., *P. fumosoreus, B. bassiana, Chaetomium* sp., *Gliocladium* sp., *Aurebasidium* sp., *Dabaryomyces* sp., *Exophilia* sp., *Ampelomyces* sp., *Saccharomyces,* and *Mariannaea* sp.

Specifically exemplified herein is the use of the bacterium *Pseudomonas syringae pv. tagetis*. There are numerous other toxigenic and non-toxigenic microbes which can also be applied using the teachings of the present invention. For example, other pathovars in the species *Pseudomonas syringae* can be used according to the subject invention. The methods of the subject invention can also be used to apply any one of a wide range of bacterial and fungal agents which produce compounds which have direct or indirect plant hormonal effects such as, but not limited to, stem elongation, sucker suppression, and leaf or fruit drop or retention.

Another embodiment of the subject invention involves the application of microbes which are competitive or antagonistic to plant pathogens. Similarly, microbes which are either toxic or repellent, or which otherwise interfere with insects or mites, can be applied using the teachings of this invention. Such organisms, or their products generated in-situ, can be directly toxic to insect or mite pests, they can deter pests through taste or smell, or, alternatively, they can produce substances which attract desirable insects.

Microbes applied in the oil emulsions of the subject invention are more successfully introduced into plants than through the prior art use of silicone surfactants. In the systems described herein, the class and concentration of emulsifier used to achieve the emulsion is not sufficient alone to allow significant infection or colonization of the plant by the desired microbe.

In addition, microbes applied according to the subject invention are capable of use outside of their "natural" host range—the set of plant species on which they would grow or function without the artificial inoculation described here.

Detailed Disclosure of the Invention

The subject invention pertains to the use of a hydrophobic oil emulsified in water as the carrier to introduce live microbes onto plants such that the microbes will colonize the plant. The oil used according to the subject invention can be, for example, paraffinic oils, or vegetable or animal based oils. Other oils having the physical characteristics of these oils could also be used. Typically, the paraffinic oils are known to contain C_{18} to about C_{24} saturated hydrocarbons as well as cyclic

alkanes and some saturated compounds. The skilled artisan with the benefit of this description can utilize these components as described herein. The vegetable and animal oils are typically triglycerides. Hydrophobic derivatives of these compounds, such as methyl esters of fatty acids can also be used as described herein.

The microbes useful according to the subject invention can be, for example, bacteria, fungi, or yeast. Microbes applied as described herein are able to "infect" or "colonize" the plant in such a way that the microbes remain viable and are able to carry out the biological processes which make them desirable horticultural tools.

According to the subject invention, desirable microbes (such as bioherbicides, biofungicides, bioinsecticides, and microbial plant growth-regulating agents) are applied to the aerial portions (areas above ground) of plants as a suspension in an emulsified mixture of at least one hydrophobic oil and water. The emulsion comprises water, the hydrophobic oil(s), and an amount of emulsifier(s), sufficient to create a stable water/oil emulsion. The hydrophobic oil will typically be present at a concentration between about 0.5% and about 10%. Most preferably the concentration of oil will be about 1% to about 5%. The concentration of emulsifier can be, for example, about 0.02% to about 2%. The concentration of emulsifier will be sufficient to create an emulsion.

Hydrophobic oils useful for the emulsions of the subject invention are commonly available in the form of various "spray oils." Unlike silicone surfactants, these oil emulsions do not have low surface tensions and are not known for entering stomata. For chemical herbicides, they are known to alter the waxy cuticle of the leaf or stem and thus enhance the movement of a chemical across that barrier. In the case of chemicals, the oil emulsion is aiding in the movement of chemicals that are in true solution in the oil or water fraction of the spray and in the wax layer of the plant. Obviously, a particulate microbe as used according to the subject invention cannot be affected through solution mechanisms, yet, surprisingly, successful plant inoculation occurs when microbes are suspended in such emulsions as described herein.

Bacteria which can be applied according to the subject invention include pesticidal bacteria such as *Bacillus thuringiensis* (*B.t.*) or recombinant microbes which express pesticidal *B.t.* δ-endotoxins. Various pesticidal *B.t.* isolates are well known to those skilled in the art and are described in, for example, U.S. Patent Nos. 5,281,530; 4,849,217; 5,064,648; 5,098,705; 5,045,469; 5,164,180; 5,188,960; 5,350,577; 5,298,245; and 5,286,486. Recombinant bacteria which express *B.t.* toxins are also well known to those skilled in the art. General techniques for applying live biocontrol agents are described in, for example, U.S. Patent No. 5,281,532.

Bacterial agents which can be used according to the subject invention further include bacterial herbicides. Representative of these bacteria agents are various *Pseudomonads* and

Xanthomonas isolates. See, for example, U.S. Patent Nos. 5,192,541 and 5,271,932 and EP 0 620 972.

Bacterial colonization can also be used to prevent or reduce frost damage or to control fruit russetting. See, for example, U.S. Patent No. 5,877,438.

Fungi which can be applied according to the subject invention include herbicidal fungi and pesticidal fungi. U.S. Patent No. 3,999,973 to Templeton teaches the use of *Colletotrichum malvarum* as a mycoherbicide for controlling prickly sida (*Sida spinosa L.*) U.S. Patent No. 4,606,751 to Van Dyke *et al.* describes the use of *Bipolaris sorghicola* as a mycoherbicide for controlling the growth of Johnsongrass (*Sorghum haepense*). U.S. Patent No. 4,636,386 to Anderson *et al.*, discloses the use of a strain of *Alternaria zinniae* for use in controlling the growth of Italian thistle (*Carduus pycnocephalus*). *Fusarium roseum* Culmorum may be used for the mycoherbicidal control of *Hydrilla verticillata* (U.S. Patent No. 4,263,036 to Charudattan). For further discussion of the use of mycoherbicides, see R. Charudattan, "The Use of Natural and Genetically Altered Strains of Pathogens for Weed Control" in M.A. Hoy and D.C. Herzog (eds.), *Biological Control in Agricultural IPM Systems*, N.Y.: Academic Press, 1985, pp. 347-372 and G.E. Templeton, "Biological Control of Weeds," *Amer. J. Alternative Agr.* 3:69-72 (1989).

In some instances, chemical herbicides may be used in combination with a mycoherbicide to enhance control of weeds that are tolerant to the chemical herbicide or the mycoherbicide. *E.g.*, R.A. Klerk *et al.*, Integration of a Microbial Herbicide into Weed and Pest Control Programs in Rice (*Oryza sativa*), *Weed Sci.* 33:95-99 (1985); U.S. Patent No. 4,755,208 to Riley and Walker. Mycoherbicides have also been used in combination with chemical herbicides where both attack the same weeds. See, e.g., U.S. Patent No. 4,776,873 to Caulder *et al.*

Pesticidal fungi useful according to the subject invention include *P. fumosoreus* which controls whiteflies. See, for example, U.S. Patent No. 4,942,030.

The method of the subject invention can also be used to alter the microflora of plant surfaces. This may be desirable for a variety of reasons including, for example, the exclusion of ice nucleating microbes which facilitate frost damage. Organisms which can be successfully established in/on plants through the method of the subject invention can include those which have been intentionally selected, modified, recombinantly transformed, mutated, or otherwise changed to be able to express it-situ a desirably function.

Following are examples which illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1

Zinnias were grown to two true leaf stage in the greenhouse. *Pseudomonas syringae* pv *tagetis* cells were diluted to 1 x 10^8 cfu ml^{-1} in deionized water.

Treatments were applied by a track sprayer. The track sprayer was at full speed (6 mph), 35 psi with 8008 EVS nozzle.

After treatment, plants were grown in a CONVIRON E15 growth chamber set at 12 hour days at 24C and 12 hour nights at 19C. Plants were rated at 12 days after treatment. Two sets of four plants were used for each treatment.

Table 1

1 X 10^8 cfu/ml, rated 12 DAT for % infected

Treatment	% oil in emulsion	add emulsifier (% in final spray solution)	result (% plants infected)
SILWET	0.1		65
Sun 6E (emulsified paraffinic oil)	2		29
Sun 6E (emulsified paraffinic oil)	4		42
Sun 6E (emulsified paraffinic oil)	6		67
Crude corn oil	2	Trycol 6964, .1%	48
Crude corn oil	4	Trycol 6964, .2%	92
Crude corn oil	6	Trycol 6964, .3%	82
Sun 6N paraffinic oil	2	Trycol 6964, .1%	27
Sun 6N paraffinic oil	4	Trycol 6964, .2%	50
Sun 6N paraffinic oil	6	Trycol 6964, .3%	87
Sun 11N paraffinic oil	2	Trycol 6964, .1%	12
Sun 11N paraffinic oil	4	Trycol 6964, .2%	71
Sun 11N paraffinic oil	6	Trycol 6964, .3%	100
UFO (emulsifiable paraffinic oil)	2		59
UFO (emulsifiable paraffinic oil)	4		86
UFO (emulsifiable paraffinic oil)	6		88

Table 2		
Treatment	Rate	% severely infected
SILWET L-77	0.1	8
Kinetic (commercial adjuvant including some silicon surfactant)	0.1	0
Kinetic (commercial adjuvant including some silicon surfactant)	0.2	5
AGRIDEX (emulsifiable paraffinic oil)	2	0
AGRIDEX (emulsifiable paraffinic oil)	4	12
AGRIDEX (emulsifiable paraffinic oil)	4	68
Soydex (emulsifiable vegetable oil based spray additive)	2	0
Soydex (emulsifiable vegetable oil based spray additive)	4	53
Soydex (emulsifiable vegetable oil based spray additive)	6	93
UFO (emulsifiable paraffinic oil)	4	5
UFO (emulsifiable paraffinic oil)	5	0
UFO (emulsifiable paraffinic oil)	6	14

Table 3		
Treatment	Rate	Result (% severely infected)
SILWET L-77	0.1	0
Sun 6E paraffinic oil	2.2	0
Sun 6E paraffinic oil	4.4	9
Sun 6E paraffinic oil	6.6	47
UFO	2.2	8
UFO	4.4	0
UFO	6.6	23
Trycol 6964 (emulsifier alone)	.05	0
Trycol 6964 (emulsifier alone)	0.1	0
Trycol 6964 (emulsifier alone)	0.2	0
Volck Oil Spray	2.2	0
Volck Oil Spray	4.4	17
Volck Oil Spray	6.6	38
Greenlight Dormant Spray Oil	2.2	0
Greenlight Dormant Spray Oil	4.4	38
Greenlight Dormant Spray Oil	6.6	61
CMR Herbicide Activator	1.1	0
CMR Herbicide Activator	2.2	6
CMR Herbicide Activator	3.3	90

Treatments using SILWET L-77 were ineffective, whereas treatments containing oil were effective in inoculating bacteria into Zinnias. TRYCOL 6964 (a common emulsifier for emulsifying oil in water) did not allow infection of the Zinnias.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

References

U.S. Patent No. 4,776,873.

U.S. Patent No. 4,755,208.

U.S. Patent No. 4,755,207.

U.S. Patent No. 5,256,627.

U.S. Patent No. 5,393,728.

Roberts *et al.*, U.S. Patent No. 5,077,045.

U.S. Patent No. 5,192,541.

Zidak, N.K, P.A. Backman (1990) "The use of surfactants to facilitate infection of leaves by bacterial plant pathogens: implications for biological weed control," *Phytopathology* abstract A466 pg. 1016.

U.S. Patent No. 5,281,530.

U.S. Patent No. 4,849,217.

U.S. Patent No. 5,064,648.

U.S. Patent No. 5,098,705.

U.S. Patent No. 5,045,469.

U.S. Patent No. 5,164,180.

U.S. Patent No. 5,188,960.

U.S. Patent No. 5,350,577.

U.S. Patent No. 5,298,245.

U.S. Patent No. 5,286,486.

U.S. Patent No. 5,281,532.

U.S. Patent No. 5,192,541.

U.S. Patent No. 5,271,932.

European Patent No. EP 0 620 972.

U.S. Patent No. 5,877,438.

Templeton, U.S. Patent No. 3,999,973.

Van Dyke *et al.*, U.S. Patent No. 4,606,751.

Anderson *et al.*, U.S. Patent No. 4,636,386.

Charudattan, U.S. Patent No. 4,263,036.

Charudattan, R. (1985) "The Use of Natural and Genetically Altered Strains of Pathogens for Weed Control," in *Biological Control in Agricultural IPM Systems*, M.A. Hoy and D.C. Herzog (eds.), N.Y.: Academic Press, pp. 347-372.

Templeton, G.E. (1989) "Biological Control of Weeds," *Amer. J. Alternative Agr.* 3:69-72.

Klerk, R.A., *et al.* (1985) "Integration of a Microbial Herbicide into Weed and Pest Control Programs in Rice (*Oryza sativa*)," *Weed Sci.* 33:95-99.

Riley and Walker, U.S. Patent No. 4,755,208.

Caulder *et al.*, U.S. Patent No. 4,776,873.

U.S. Patent No. 4,942,030.

Claims

1. A method of introducing live microbes onto aerial portions of plants such that said microbes will colonize and occupy said surfaces, said method comprises applying an oil-in-water emulsion to said surfaces wherein said emulsion comprises:

a) live microbes,

b) a hydrophobic oil in an amount effective to facilitate colonization of said plant surface by said microbe, and

c) water.

2. The method, according to claim 1, wherein said emulsion further comprises a emulsifier.

3. The method, according to claim 2, wherein said emulsifier is present at a concentration of between about 0.02 and about 2%.

4. The method, according to claim 1, wherein said oil is present in a concentration of between about 0.5 and about 10%.

5. The method, according to claim 1, wherein said hydrophobic oil is selected from the group consisting of plant and animal triglyceride oils, hydrocarbon oils such as paraffinic oils which are of petroleum origin, and derivatives of said triglyceride oils and hydrocarbon oils thereof.

6. The method, according to claim 6, wherein said derivative is a methyl ester of a fatty acid.

7. The method, according to claim 1, wherein the hydrophobic oil is selected from the group consisting of Soydex, Argridex, Sun11E, UFO, Volck Spray Oil, Greenlight Dormant Spray Oil, CMR Herbicide Activator, Linseed Oil, and methyl esterss of soybean fatty acids.

8. The method, according to claim 1, wherein said microbe is a fungus.

9. The method, according to claim 1, wherein said microbe is a bacterium.

10. The method, according to claim 1, wherein said microbe is a yeast.

11. The method, according to claim 1, wherein said microbe is a pesticide.

12. The method, according to claim 12, wherein said microbe expresses a *Bacillus thuringiensis* toxin.

13. The method, according to claim 13, wherein said microbe is a *Bacillus thuringiensis* isolate.

14. The method, according to claim 13, wherein said microbe is a recombinant host transformed to express a *Bacillus thuringiensis* toxin.

15. The method, according to claim 1, wherein said microbe modulates plant growth or development.

16. The method, according to claim 1, wherein said microbe is applied in order to control unwanted vegetation.

17. The method, according to claim 17, wherein said unwanted vegetation is selected from the group consisting of Canada thistle, cocklebur, artichoke thistle, star thistle, bull thistle, mustard, foxtail, and ragweed.

18. The method, according to claim 17, wherein said microbe is selected from the group consisting of *Xanthomonas campestris* and *Pseudomonas syringae*.

19. The method according to claim 19, wherein said microbe is *Pseudomonas syringae pv. tagetis*.

20. The method, according to claim 1, wherein said microbe is selected from the group consisting of *Bacillus* sp., *Pseudomonas* sp., *Xanthomonas*, *Trichoderma* sp., *Erwinia* sp., *Pichia* sp., *Candida* sp., *Cryptococcus* sp., *Talaromyces* sp., *P. fumosoreus*, *B. bassiana*, *Chaetomium* sp., *Gliocladium* sp., *Aurebasidium* sp., *Dabaryomyces* sp., *Exophilia* sp., *Ampelomyces* sp., *Saccharomyces*, and *Mariannaea* sp.

21. An oil-in-water emulsion for applying living microbes to an aerial plant surface comprising:

a) a live microbe,

b) an oil in an amount effective to allow the microbe to colonize an aerial plant surface when the oil-in-water composition is applied to plants as a foliar spray, and

c) water.

22. The oil-in-water emulsion composition, according to claim 22, wherein said emulsion further comprises a emulsifier.

23. The oil-in-water emulsion composition, according to claim 23, wherein said emulsifier is present at a concentration of between about 0.02 and about 2%.

24. The oil-in-water emulsion composition, according to claim 22, wherein said oil is present in a concentration of between about 0.5 and about 10%.

25. The oil-in-water emulsion composition, according to claim 22, wherein said hydrophobic oil is selected from the group consisting of plant and animal triglyceride oils, hydrocarbon oils such as paraffinic oils which are of petroleum origin, and derivatives of said triglyceride oils and hydrocarbon oils thereof.

26. The oil-in-water emulsion composition, according to claim 27, wherein said derivative is a methyl ester of a fatty acid.

27. The oil-in-water emulsion composition, according to claim 22, wherein the hydrophobic oil is selected from the group consisting of Soydex, Argridex, Sun11E, UFO, Volck Spray Oil, Greenlight Dormant Spray Oil, CMR Herbicide Activator, Linseed Oil, and methyl esterss of soybean fatty acids.

28. The oil-in-water emulsion composition, according to claim 22, wherein said microbe is a fungus.

29. The oil-in-water emulsion composition, according to claim 22, wherein said microbe is a bacterium.

30. The oil-in-water emulsion composition, according to claim 22, wherein said microbe is a yeast.

31. The oil-in-water emulsion composition, according to claim 22, wherein said microbe is a pesticide.

32. The oil-in-water emulsion composition, according to claim 33, wherein said microbe expresses a *Bacillus thuringiensis* toxin.

33. The oil-in-water emulsion composition, according to claim 34, wherein said microbe is a *Bacillus thuringiensis* isolate.

34. The oil-in-water emulsion composition, according to claim 34, wherein said microbe is a recombinant host transformed to express a *Bacillus thuringiensis* toxin.

35. The oil-in-water emulsion composition, according to claim 22, wherein said microbe modulates plant growth or development.

36. The oil-in-water emulsion composition, according to claim 22, wherein said microbe is a herbicide.

37. The oil-in-water emulsion composition, according to claim 38, wherein said microbe is selected from the group consisting of *Xanthomonas campestris* and *Pseudomonas syringae*.

38. The oil-in-water emulsion composition, according to claim 39, wherein said microbe is *Pseudomonas syringae pv. tagetis*.

39. The oil-in-water emulsion composition, according to claim 22, wherein said microbe is selected from the group consisting of *Bacillus* sp., *Pseudomonas* sp., *Trichoderma* sp., *Erwinia* sp., *Pichia* sp., *Candida* sp., *Cryptococcus* sp., *Talaromyces* sp., *P. fumosoreus*, *B. bassiana*, *Chaetomium* sp., *Gliocladium* sp., *Aurebasidium* sp., *Dabaryomyces* sp., *Exophilia* sp., *Ampelomyces* sp., and *Mariannaea* sp.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/09950

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A01N63/04 A01N63/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WEED SCIENCE, vol. 43, no. 3, April 1995, pages 312-317, XP002017680 GRANT H. EGLEY & C. DOUGLAS BOYETTE: "Water-Corn Oil emulsion enhances conidia germination and mycoherbicidal activity of Colletotrichum truncatum." see page 314, column 2, paragraph 4 - page 317, column 1, paragraph 1; tables 2-4 --- -/--	1-39

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 November 1996	22.11.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Klaver, J

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/09950

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CROP PROTECTION, vol. 14, no. 3, January 1995, pages 193-197, XP002017681 T. A. KLEIN, B. A. AULD & WANG FANG: "Evaluation of oil suspension emulsions of Colletotrichum orbiculare as a mycoherbicide in field trials." see page 196, column 2, paragraph 3; tables 1-6	1-39
X	US,A,4 755 207 (BANNON JAMES S) 5 July 1988 cited in the application see example 1	1-39
X	WO,A,94 19950 (COMMW SCIENT IND RES ORG ;WILLIAMSON MARY ANNA (AU); AYLWARD JAMES) 15 September 1994 See page 8, lines 24-31, page 11, 1st. paragraph and figure 2.	1-7,9, 10,15, 20-27, 29,30, 35,39
X	T. A. ANGUS & P. LUTHY IN: H. D. BURGES & N. W. HUSSEY (EDS.): "Microbial control of insects and mites" 1971 , ACADEMIC PRESS , LONDON, NEW YORK XP002017683 Ch. 28: 'Formulation of microbial insecticides.' See especially A III. B. and C. (p. 627-628).	1-7, 9-14, 20-27, 29-34,39
X	T. L. COUCH & C. M . IGNOFFO IN: H.D. BURGES (ED.): "Microbial control of pests and plant diseases 1970-1980." 1981 , ACADEMIC PRESS , NEW YORK & LONDON XP002017684 Ch. 34: 'Formulation of insect pathogens' See especially Table 1 and page 626, 2nd par.	1-7, 9-14, 20-27, 29-34,39
X	J. OF ARBORICULTURE, vol. 7, no. 9, September 1981, pages 246-251, XP002017682 W.T. JOHNSON & O. N. MORRIS: "Cold fog applications of pesticides for control of malacosoma disstria" see page 247, column 2, paragraph 3 - paragraph 4; table 1	1-7, 9-14, 20-27, 29-34,39
1 X	WO,A,95 10597 (MYCOTECH CORP) 20 April 1995 see page 6, paragraph 2; examples 9-12,16,20	21-39

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 96/09950

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A-4755207	05-07-88	NONE		
WO-A-9419950	15-09-94	AU-A-	6277694	26-09-94
		EP-A-	0688169	27-12-95
		JP-T-	8507497	13-08-96
WO-A-9510597	20-04-95	AU-A-	8015594	04-05-95
		CA-A-	2173953	20-04-95
		EP-A-	0738317	23-10-96